Supplementary information

Exploring the interactions between *Lactobacillus rhamnosus* GG and whey protein isolate for preservation of the viability of bacteria through spray drying

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Section S1. Supporting methods

S1.1. Visualization of the integrity of LGG cellular membrane using confocal laser scanning microscopy (CLSM)

The integrity of the cellular membrane of LGG in different LGG samples was examined by staining the cells with Live/Dead® BacLight™ fluorescence probe kit (L-7012, Thermofisher, USA) followed by CLSM observation. LGG cells collected from different samples were resuspended in 0.85 wt.% NaCl. Then, 3 μL fluorescence dye, which was prepared following product specification, was added to 1 mL LGG suspension. The mixture was incubated in dark at room temperature for 15 min. The stained samples were observed using an upright Leica TCS SP8 microscope (Leica Microsystems, Wetzlar, Germany).

S1.2. Measurement of calcium concentration in WPI solution

WPI was reconstituted following the method described in Section 2.1 and diluted into a series of dilutions between 0.1 and 1 wt.%. The stock solution of Ca^{2+} standard was prepared by dissolving 2.769 g $CaCl_2$ in deionized water to prepare 1 L solution, giving a Ca^{2+} concentration of 1000 mg/L. The stock solution was diluted to 10 mg/L, and the standard Ca^{2+} solutions with concentrations of 0, 2, 4 and 8 mg/L were prepared. The standards and WPI samples were measured with inductively-coupled plasma spectrometer (ICP; 710-ES, Varian China Co. Ltd., American). The results of WPI sample at appropriate dilutions were compared to the obtained standard curve to determine Ca^{2+} concentration.

Section S2. Supporting results

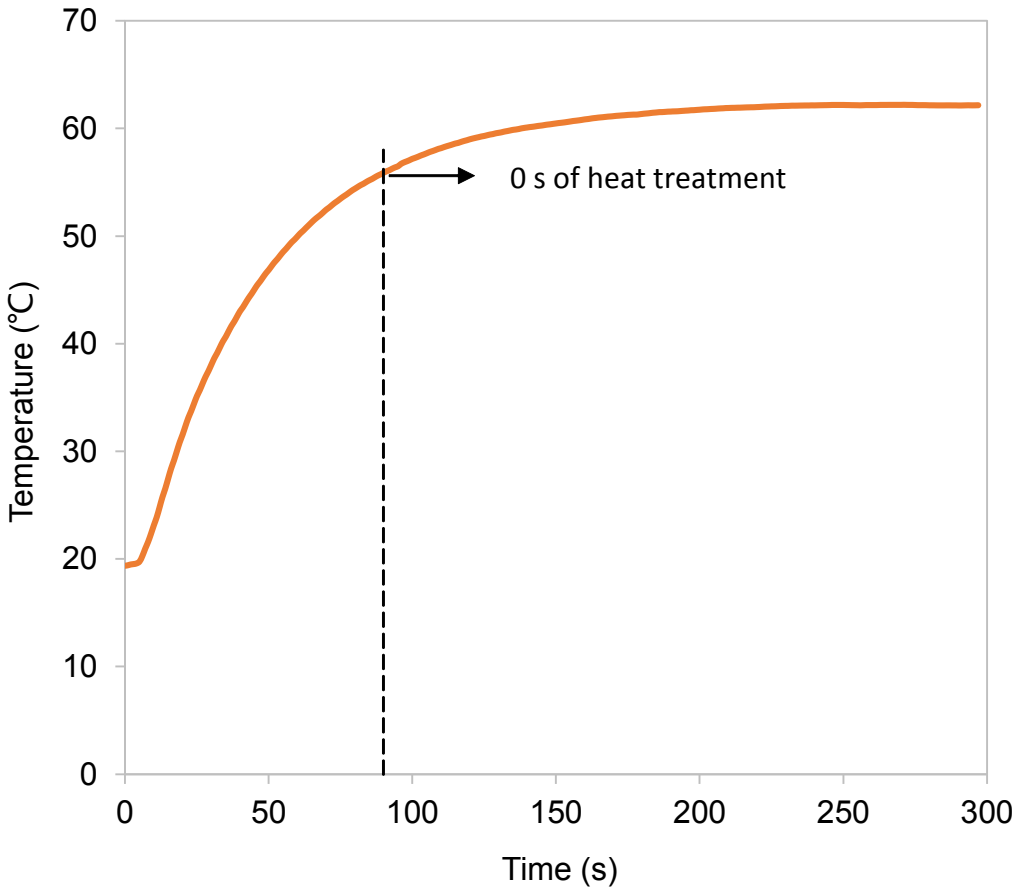

Figure S1. The temperature profile of LGG samples during heat treatment at 62 °C. The temperature measurement was performed with 1 mL of deionized water placed in a 2 mL centrifuge tube, which was then heated in a water bath at 62 °C. Changes in sample temperature were monitored with a Type-K thermocouple inserted at the center of the sample, and were recorded by a data-logger (TC-08, Pico Technology, UK).

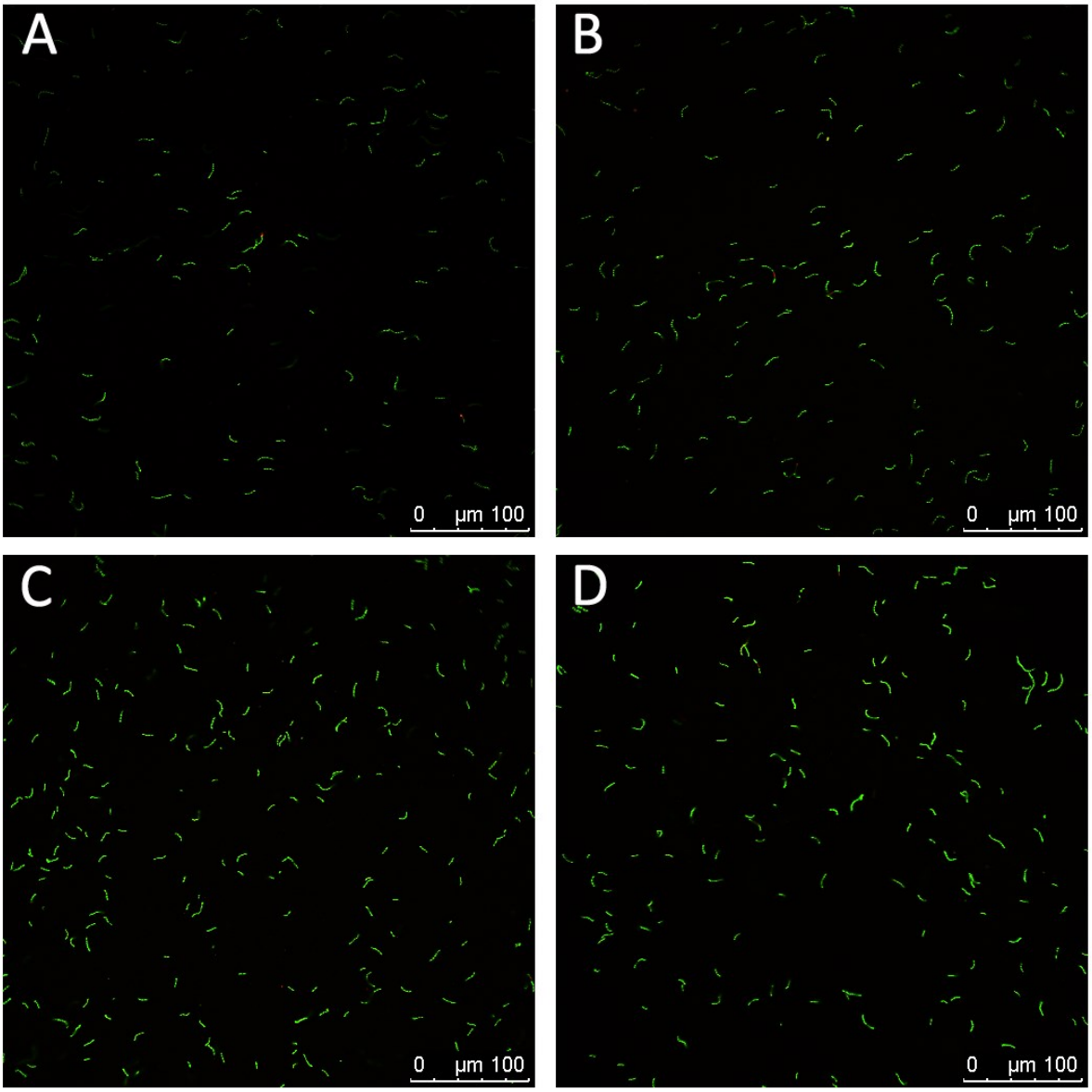

Figure S2. The integrity of cellular membrane of LGG cells after WPI adsorption treatment for 10 or 60 min. (A) LGG@0.85%NaCl; (B) LGG@10%Tre; (C) LGG-WPI_{10}@10%Tre; (D) LGG-WPI_{60}@10%Tre. All types of cell samples were stained with Live/Dead® BacLight™ kit, with green fluorescence indicating live cells with intact membrane.

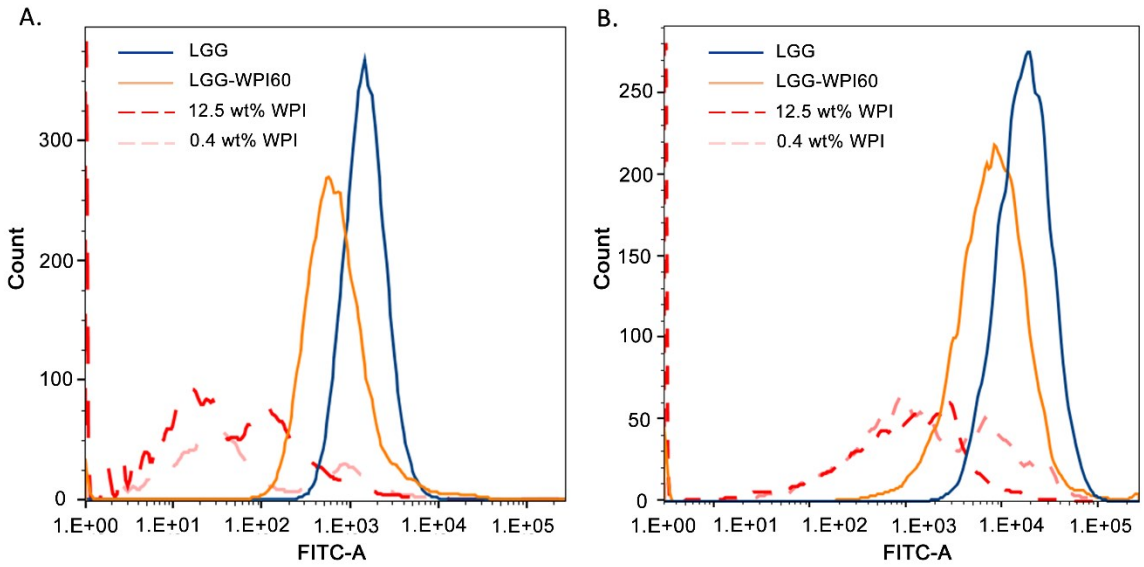

Figure S3. Flow cytometric analysis of different LGG samples with and without WPI adsorption treatment, using (A) 0.01 mg/mL FITC and (B) 0.1 mg/mL FITC to label LGG cells.